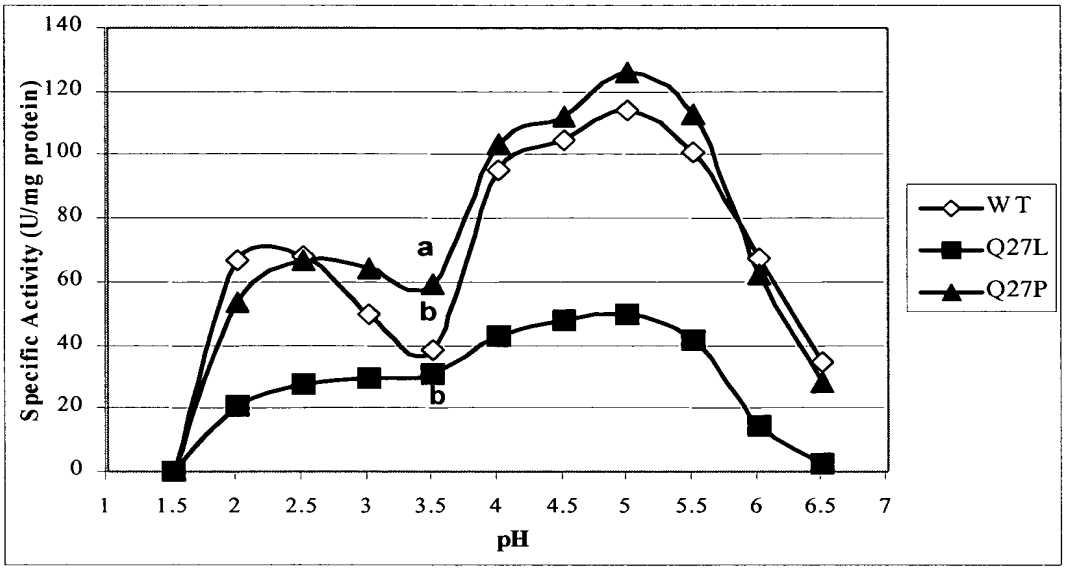
Specific Activity (U/mg protein)
140
120
100
80
60
40
20
0
1
1.5
2
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
pH
a
b
b
WT
Q27L
Q27P

*FIG. 1*

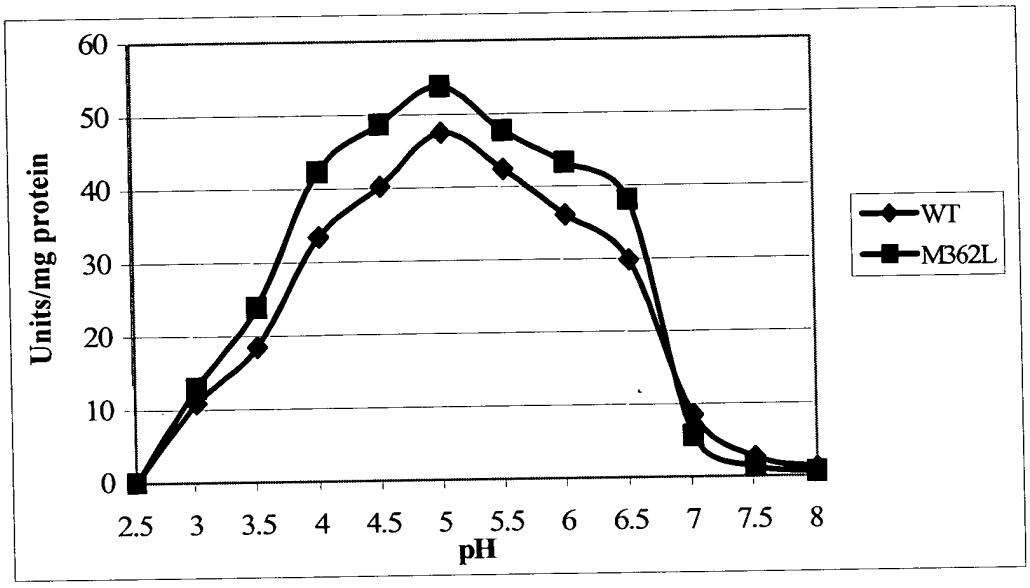
60
50
40
30
20
10
0
Units/mg protein
2.5
3
3.5
4
4.5
5
5.5
6
6.5
7
7.5
8
pH
WT
M362L

*FIG. 2*


*FIG. 3*

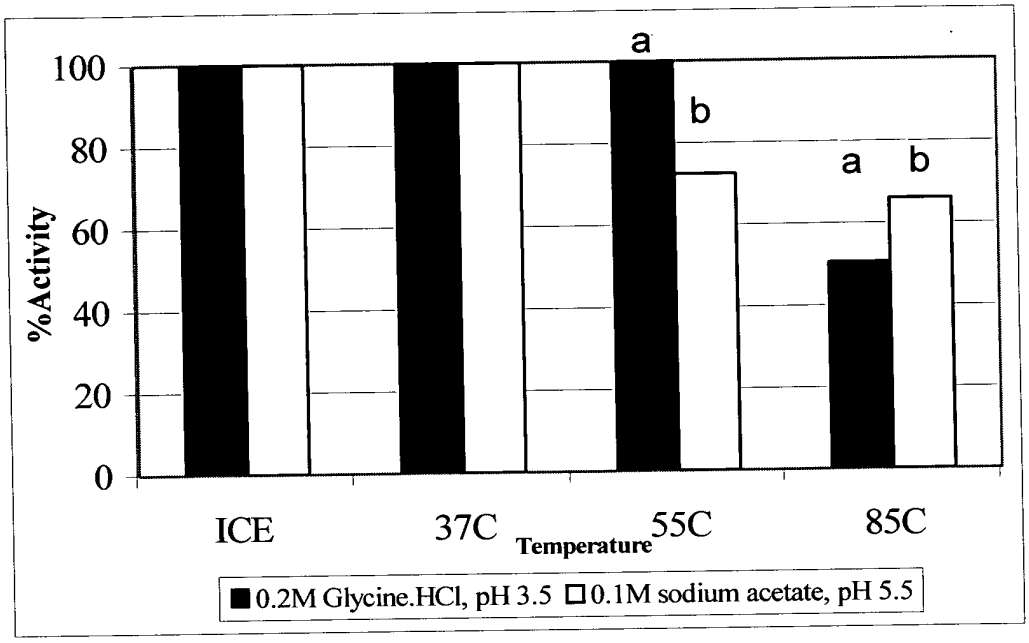
100
80
60
40
20
0
%Activity
a
b
a
b
ICE
37C
55C
85C
Temperature
0.2M Glycine.HCl, pH 3.5
0.1M sodium acetate, pH 5.5

*FIG. 4*

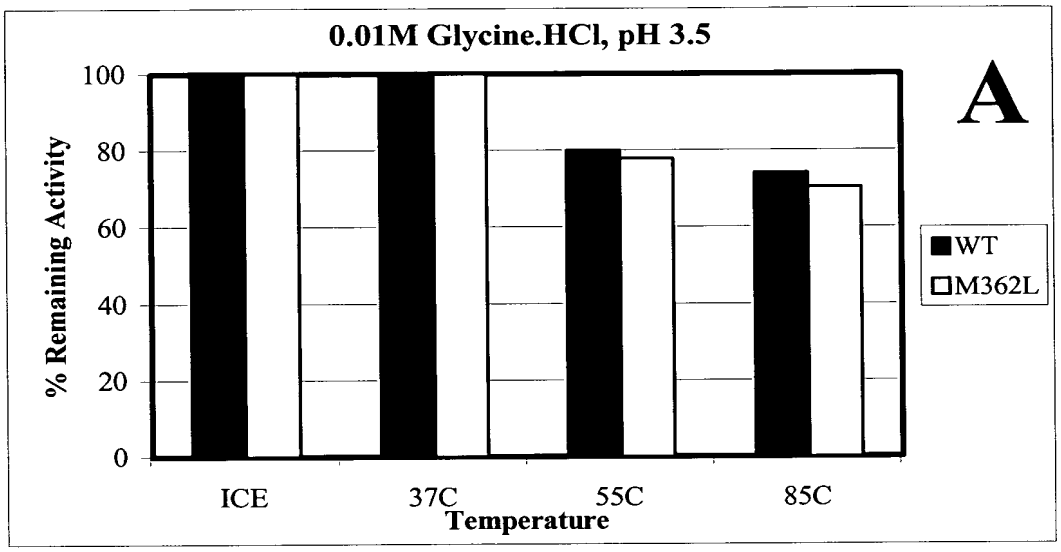
0.01M Glycine.HCl, pH 3.5
A
100
80
60
40
20
0
% Remaining Activity
ICE
37C
55C
85C
Temperature
WT
M362L

*FIG. 5A*

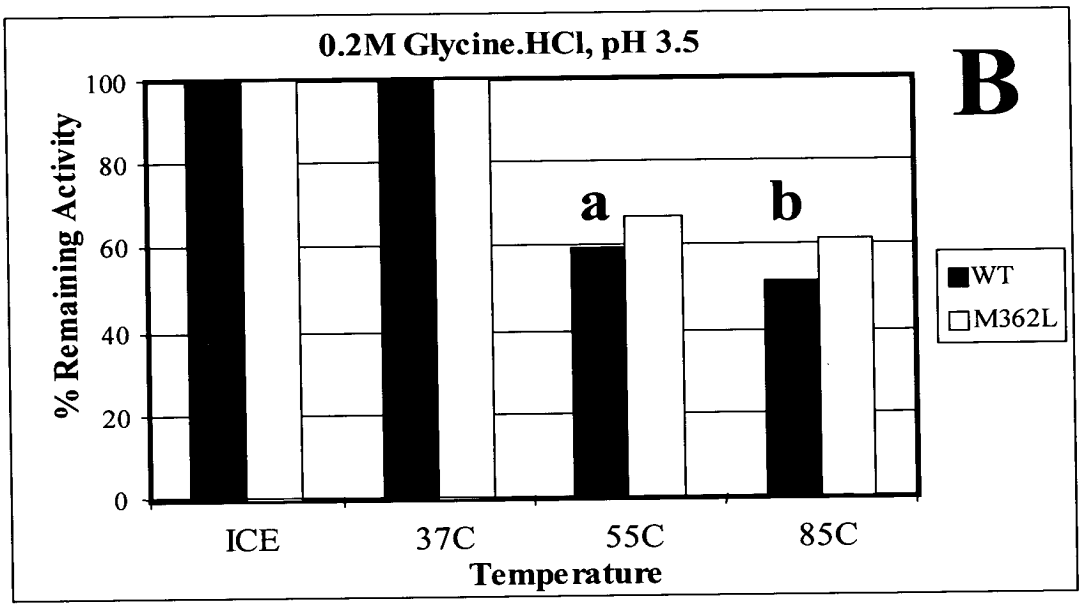
0.2M Glycine.HCl, pH 3.5
B
% Remaining Activity
100
80
60
40
20
0
a
b
ICE
37C
55C
85C
Temperature
WT
M362L

*FIG. 5B*

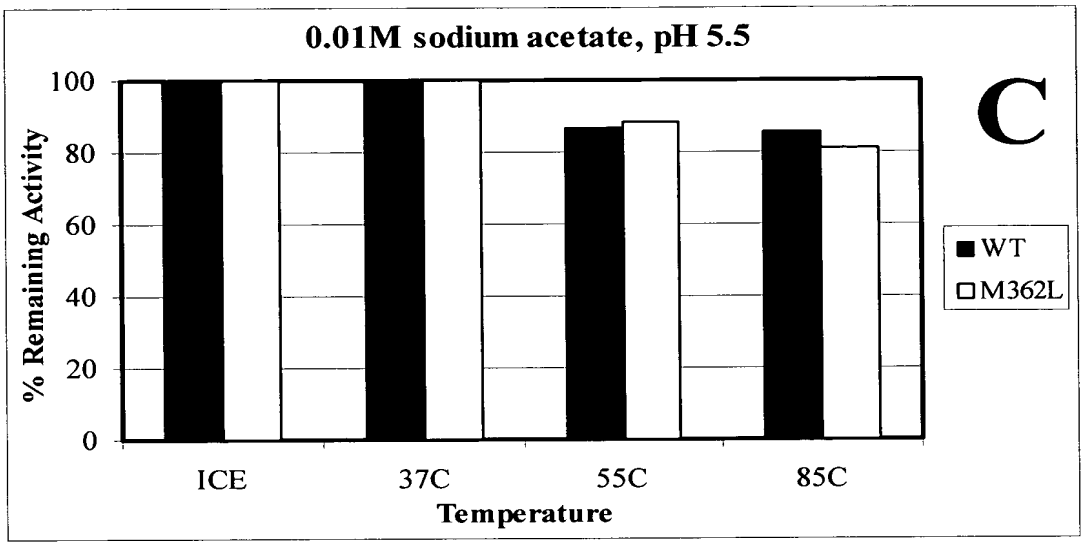
0.01M sodium acetate, pH 5.5
C
100
80
60
40
20
0
% Remaining Activity
ICE
37C
55C
85C
Temperature
WT
M362L

*FIG. 5C*

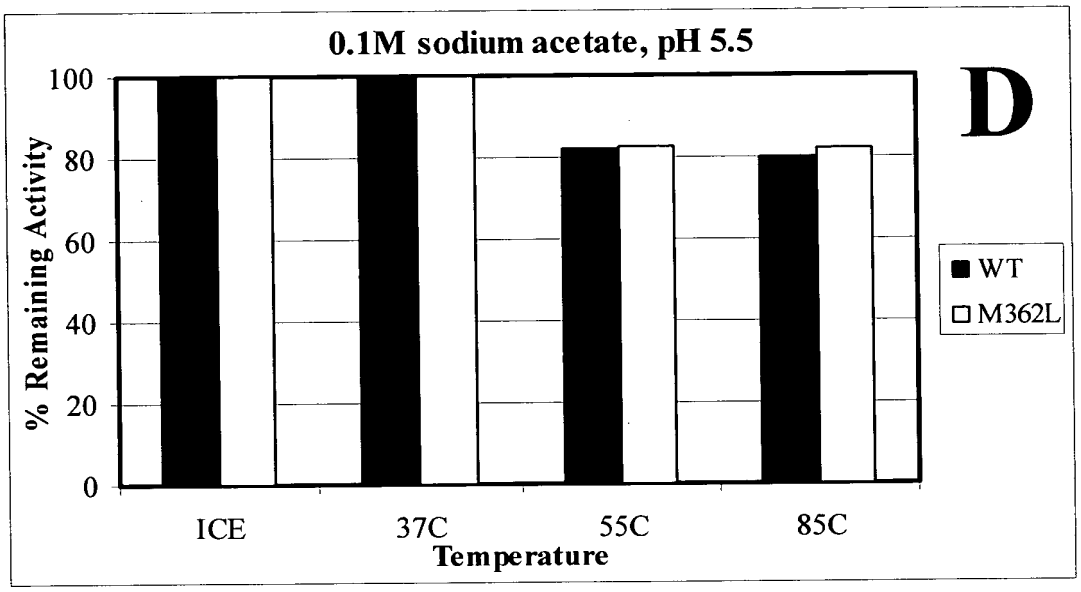
0.1M sodium acetate, pH 5.5
D
% Remaining Activity
100
80
60
40
20
0
ICE
37C
55C
85C
Temperature
WT
M362L

*FIG. 5D*

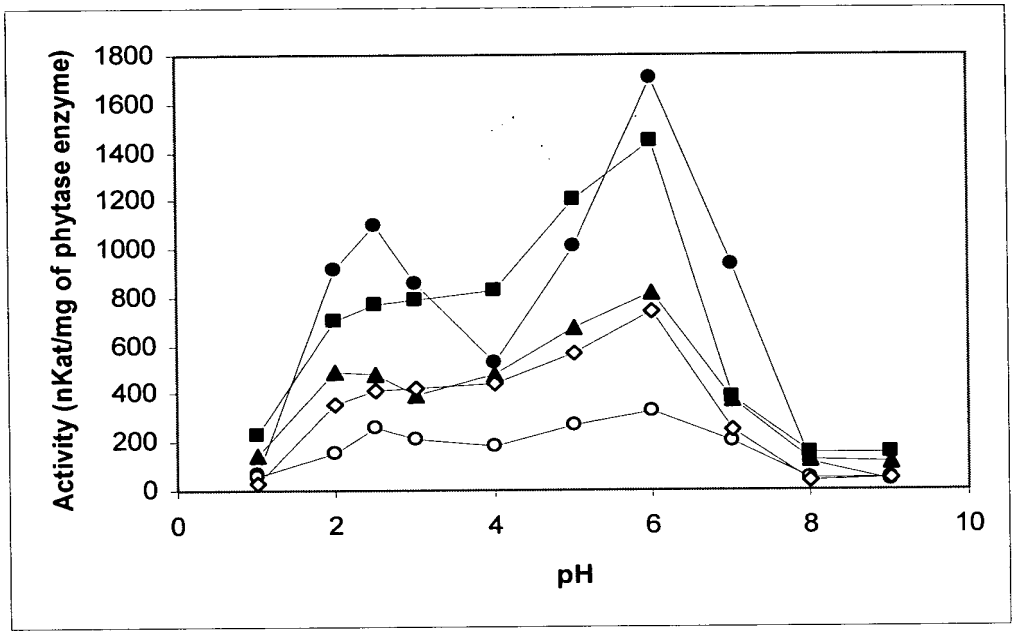
Activity (nKat/mg of phytase enzyme)
1800
1600
1400
1200
1000
800
600
400
200
0
0
2
4
6
8
10
pH

*FIG. 6*

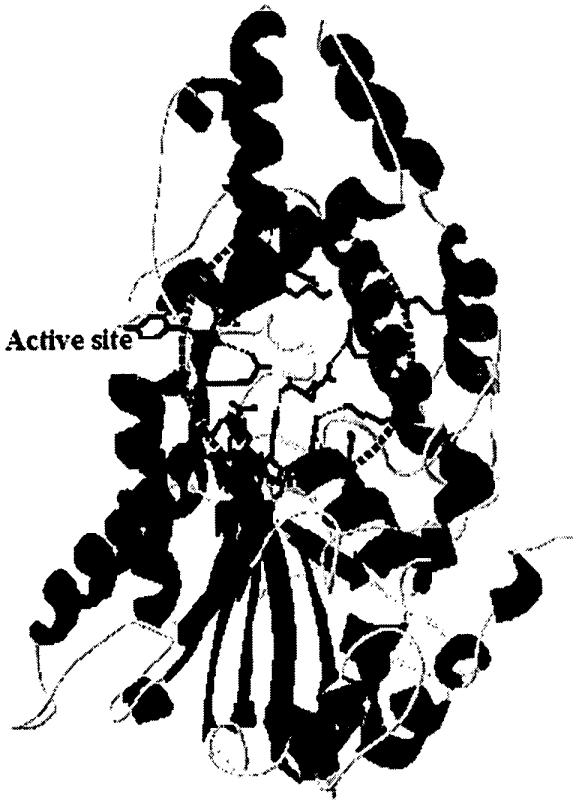
Active site

FIG. 7A

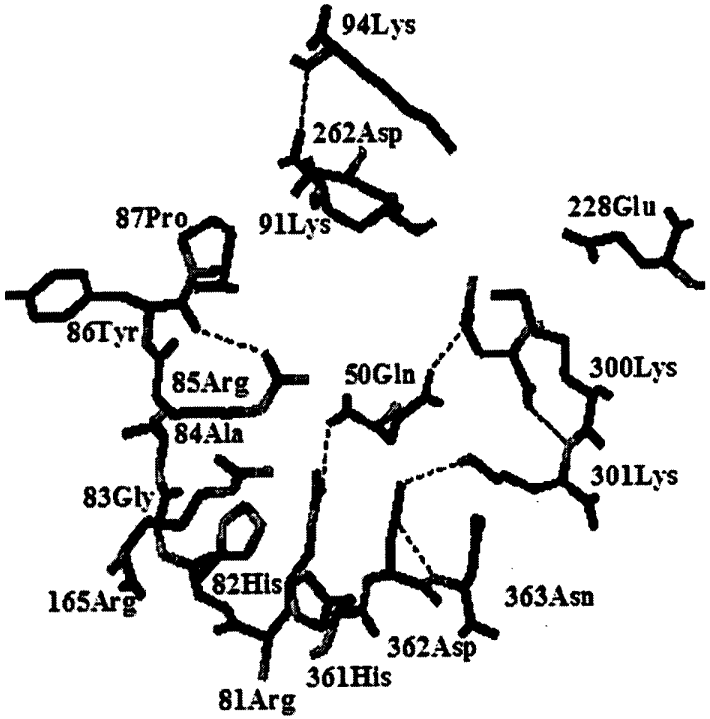
94Lys
262Asp
87Pro
91Lys
228Glu
86Tyr
85Arg
50Gln
300Lys
84Ala
301Lys
83Gly
165Arg
82His
363Asn
362Asp
361His
81Arg

FIG. 7B

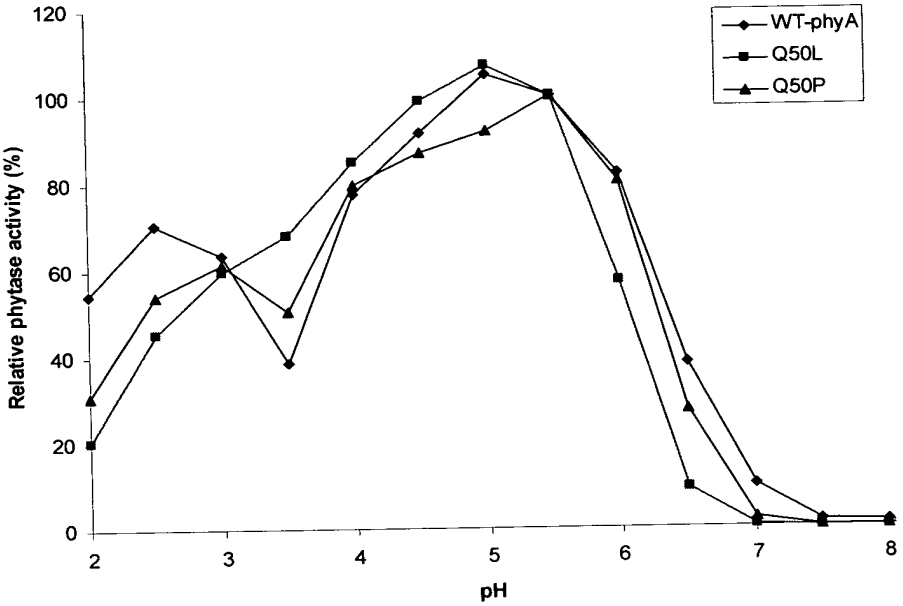
WT-phyA
Q50L
Q50P
Relative phytase activity (%)
120
100
80
60
40
20
0
2
3
4
5
6
7
8
pH

*FIG. 8A*

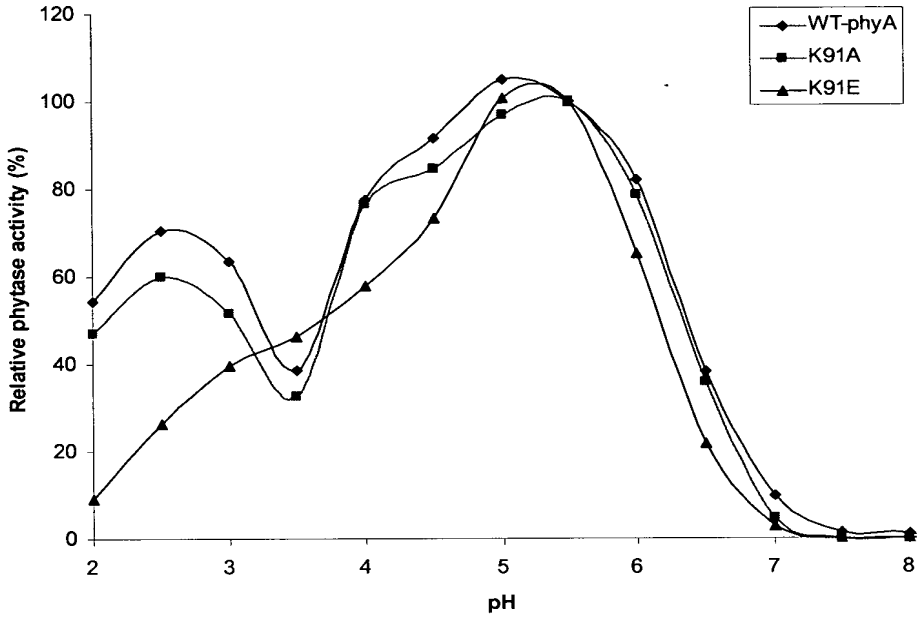
120
100
80
60
40
20
0
Relative phytase activity (%)
2
3
4
5
6
7
8
pH
WT-phyA
K91A
K91E

*FIG. 8B*

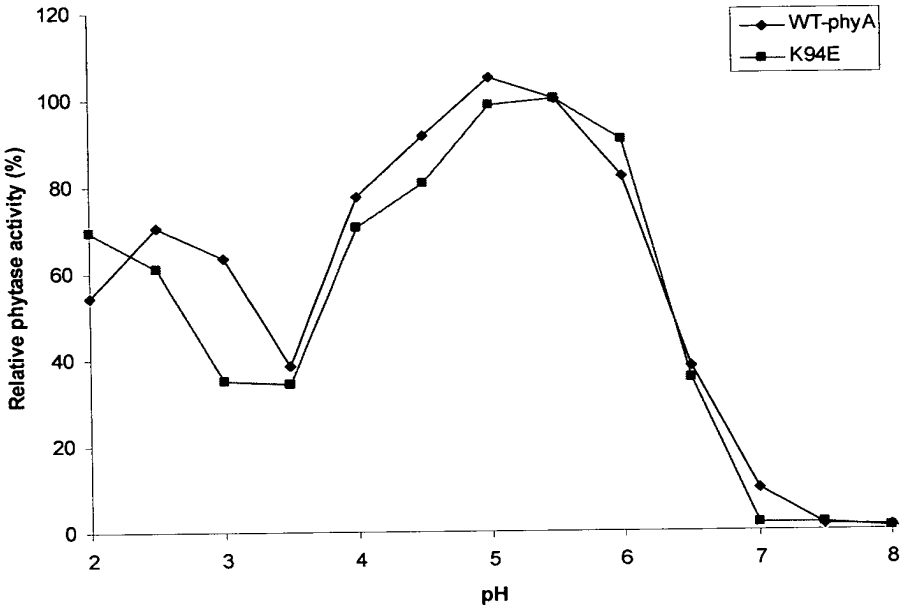
WT-phyA
K94E
Relative phytase activity (%)
120
100
80
60
40
20
0
2
3
4
5
6
7
8
pH

*FIG. 8C*

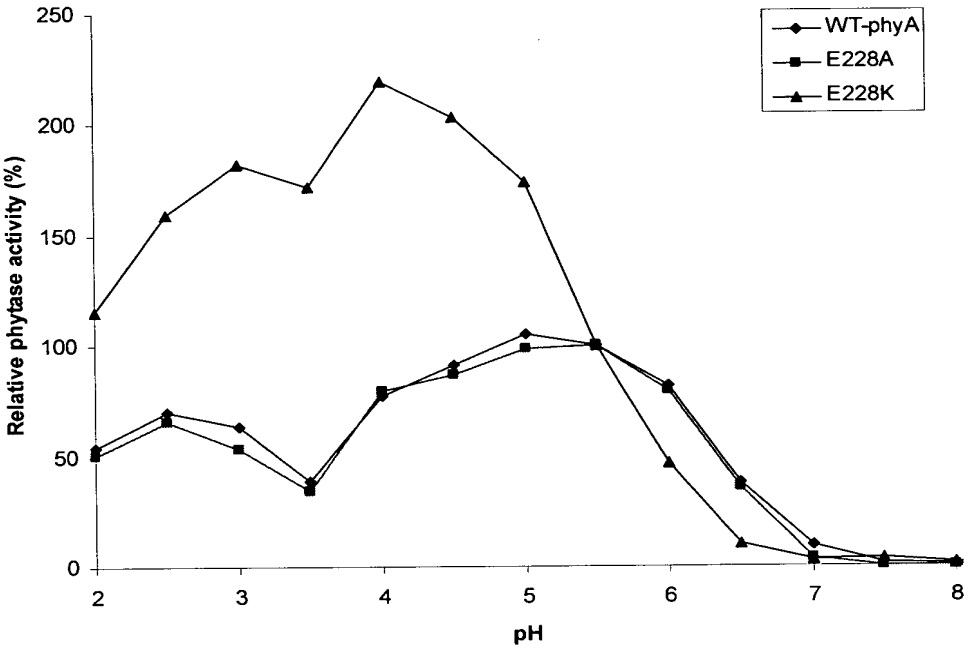
WT-phyA
E228A
E228K
Relative phytase activity (%)
250
200
150
100
50
0
2
3
4
5
6
7
8
pH

*FIG. 8D*

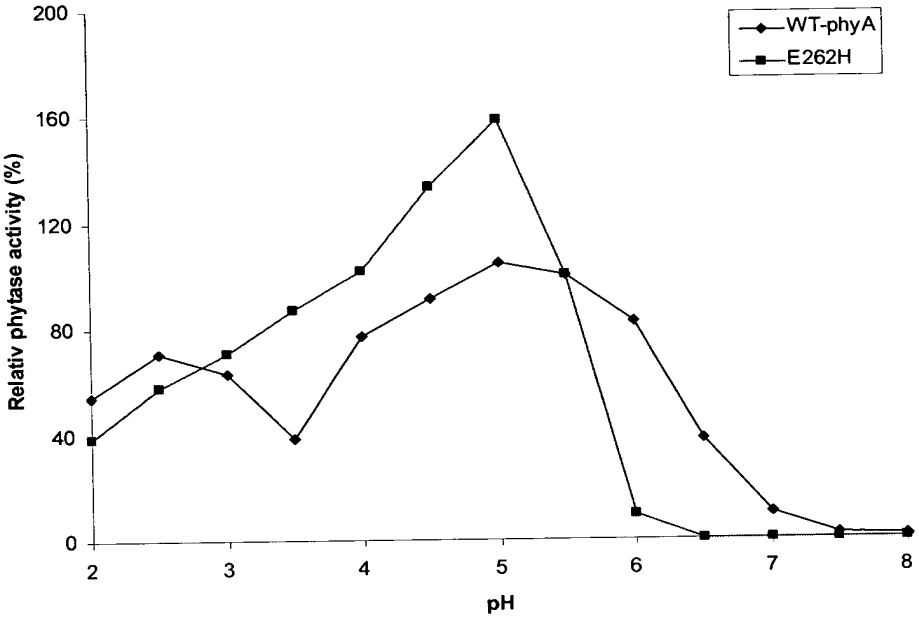
WT-phyA
E262H
Relativ phytase activity (%)
200
160
120
80
40
0
2
3
4
5
6
7
8
pH

*FIG. 8E*

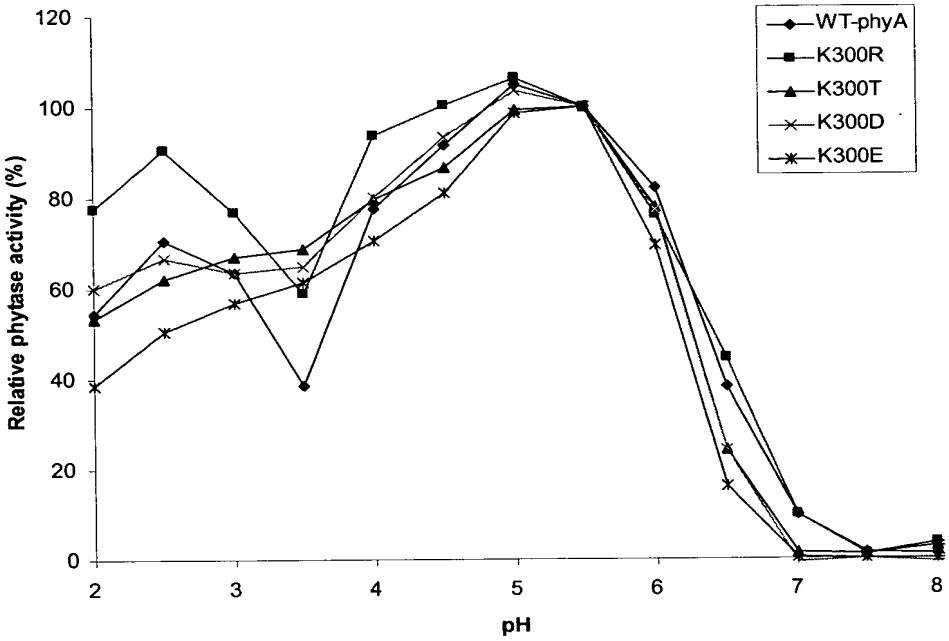
120
100
80
60
40
20
0
Relative phytase activity (%)
2
3
4
5
6
7
8
pH
WT-phyA
K300R
K300T
K300D
K300E

*FIG. 8F*

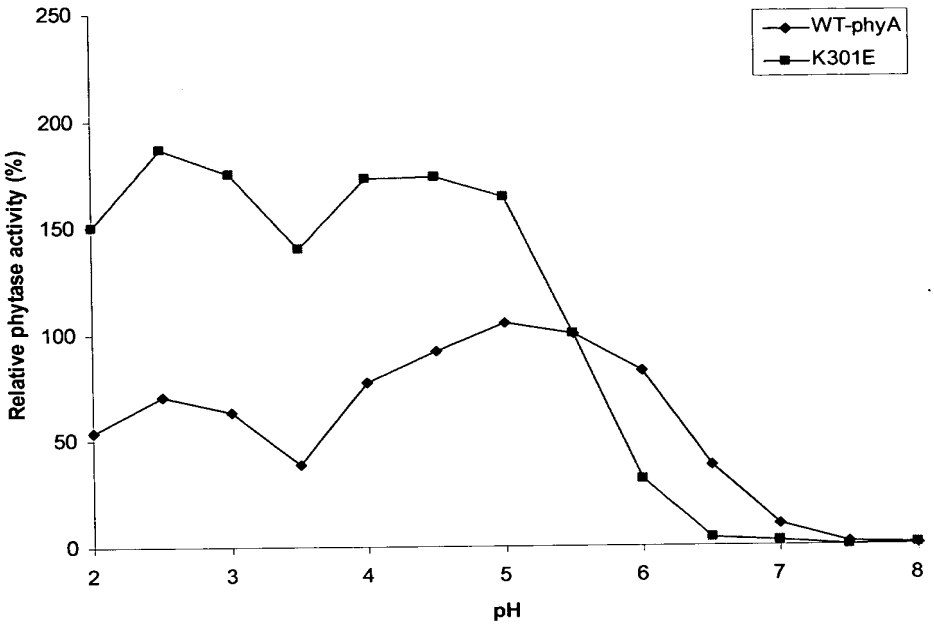
WT-phyA
K301E
Relative phytase activity (%)
250
200
150
100
50
0
2
3
4
5
6
7
8
pH

*FIG. 8G*

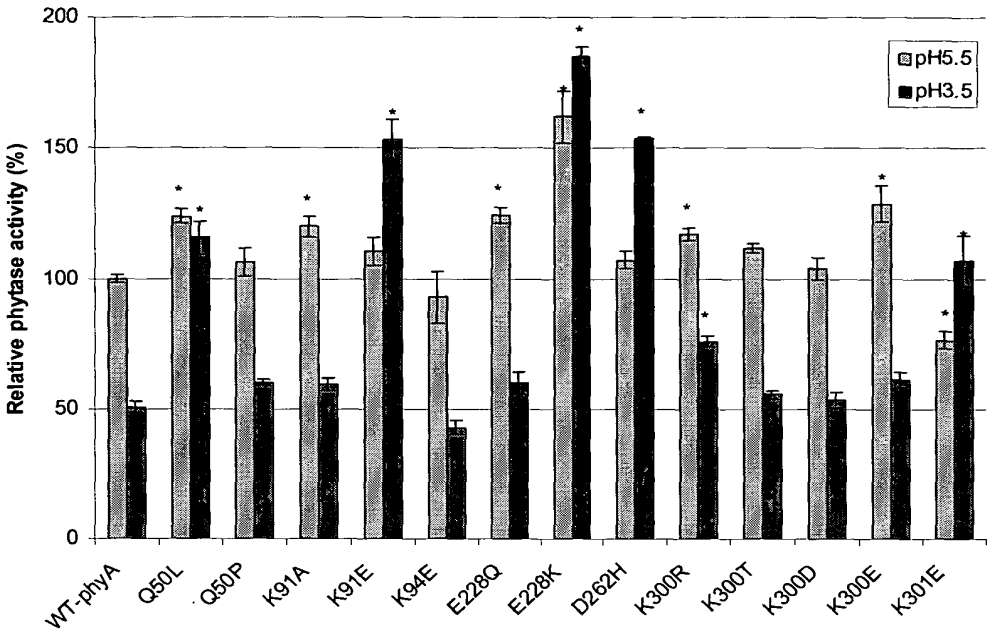
200
150
100
50
0
Relative phytase activity (%)
pH5.5
pH3.5
WT-phyA
Q50L
Q50P
K91A
K91E
K94E
E228Q
E228K
D262H
K300R
K300T
K300D
K300E
K301E

*FIG. 9*

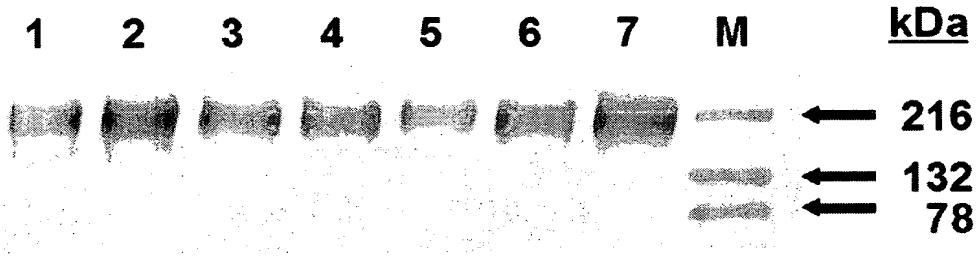


*FIG. 10A*

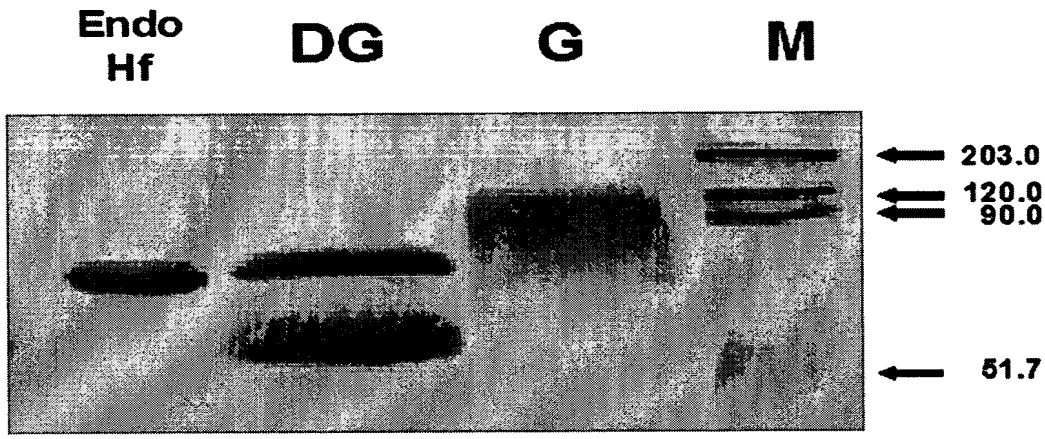


*FIG. 10B*

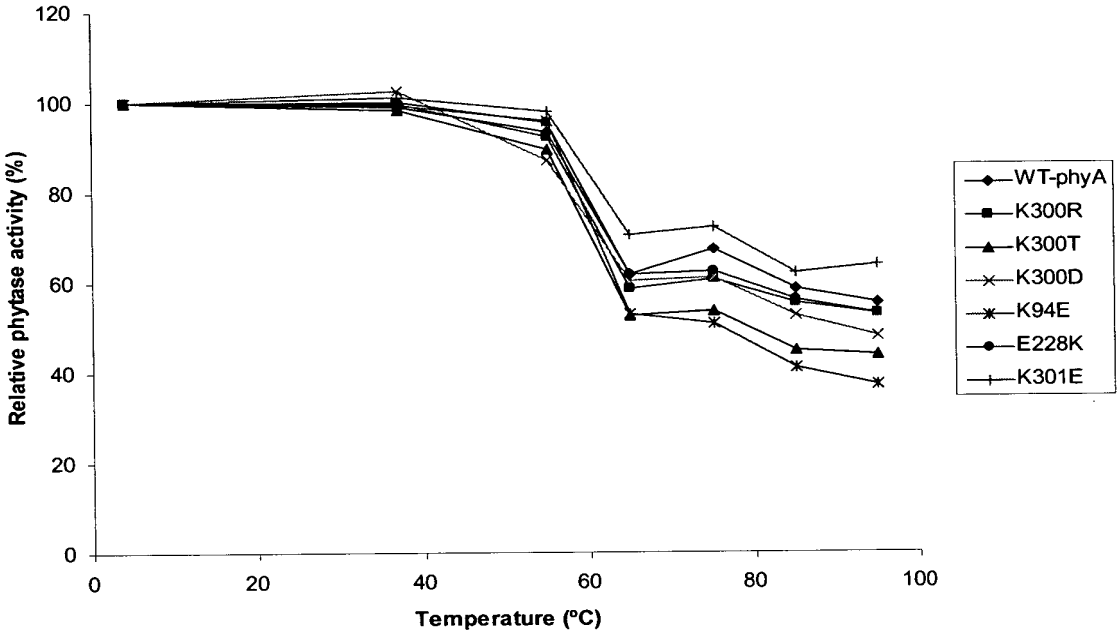
120
100
80
60
40
20
0
Relative phytase activity (%)
0
20
40
60
80
100
Temperature (°C)
WT-phyA
K300R
K300T
K300D
K94E
E228K
K301E

*FIG. 11*

1st line: Asp. terreus phytase
2nd line: Asp. niger phytase
3rd line: Asp. fumigatus phytase

```
1   MGVFVVLLSI ATLFGSTSGT ALGPRGNHSD CTSVDRGYQC EPELSHKWGL
1   MGVSAVLLPL YLLSGVTSGL AVPASRNQSS CDTVDQGYQC FSETSHLWGQ
1   MVTLTFLLSA AYLLSGRVS- AAPSSAGSKS CDTVDLGYQC SPATSHLWGQ
****                              SSS  SSSS

51  YAPYFSLQDE SPFPLDVPDD CHITFVQVLA RHGARSPTDS KTKAYAATIA
51  YAPFFSLANE SVISPEVPAG CRVTFAQVLS RHGARYPTDS KGKKYSALIE
50  YSPFFSLEDE LSVSSKLPKD CRITLVQVLS RHGARYPTSS KSKKYKKLVT
****   SSS              S SSSSSSSSSSSS SSS  HH HHHHHHHHHH

101 AIQKNATALP GKYAFLKSYN YSMGSENLNP FGRNQLQDLG AQFYRRYDTL
101 EIQQNATTFD GKYAFLKTYN YSLGADDLTP FGEQELVNSG IKFYQRYESL
100 AIQANATDFK GKFAFLKTYN YTLGADDLTP FGEQQLVNSG IKFYQRYKAL
****HHHHH         HHHH           SSSH HHHHHHHHHH HHHHH   HH

151 TRHINPFVRA ADSSRVHESA EKEVEGFQNA RQGDPHANPH QPSPRVDVVI
151 TRNIVPFIRS SGSSRVIASG KKFIEGFQST KLKDPRAQPG QSSPKTDVVI
150 ARSVVPFIRA SGSDRVIASG EKFIEGFQQA KLADPGA-TN RAAPATSVII
****H     SSSS SS HHHHHHH HHHHHHHHHH HH              SSS

201 PEGTAYNNTL EHSICTAFEA STVGDAAADN FTAVFAPAIA KRLEADLPGV
201 SEASSSNNTL DPGTCTVFED SELADTVEAN FTATFVPSIR QRLENDLSGV
199 PESETFNNTL DHGVCTKFEA SQLGDEVAAN FTALFAPDIR ARAEKHLPGV
****                HHHH   HHHHHHHHH HHH  HHHH HHHHHHH

251 QLSADDVVNL MAMCPFETVS LTDDAHTLSP FCDLFTAAEW TQYNYLLSLD
251 TLTDTEVTYL MDMCSFDTIS TSTVDTKLSP FCDLFTHDEW INYDYLQSLK
249 TLTDEDVVSL MDMCSFDTVA RTSDASQLSP FCQLFTHNEW KKYNYLQSLG
****     HHHHHH HHHHHHHHH              HHH   HHHH HHHHHHHHHH

301 KYYGYGGGNP LGPVQGVGWA NELIARLTRS PVHDHTCVNN TLDANPATFP
301 KYYGHGAGNP LGPTQGVGYA NELIARLTHS PVHDDTSSNH TLDSSPATFP
299 KYYGYGAGNP LGPAQGIGFT NELIARLTRS PVQDHTSTNS TLVSNPATFP
****H             HH    HHH HHHHHHHH            H HHH

351 LNATLYADFS HDSNLVSIFW ALGLYNGTKP LSQTTVEDIT RTDGYAAAWT
351 LNSTLYADFS HDNGIISILF ALGLYNGTKP LSTTTVENIT QTDGFSSAWT
349 LNATMYVDFS HDNSMVSIFF ALGLYNGTEP LSRTSVESAK ELDGYSASWV
****   SSSSSSS  HHHHHHHHHH H                  HHHH

401 VPFAARAYIE MMQCRAEKQP LVRVLVNDRV MPLHGCAVDN LGRCKRDDFV
401 VPFASRLYVE MMQCQAEQEP LVRVLVNDRV VPLHGCPVDA LGRCTRDSFV
399 VPFGARAYFE TMQCKSEKEP LVRALINDRV VPLHGCDVDK LGRCKLNDFV
****   SSSSSSS SSSSS    S SSSSSSS SS S             SSSHHHH

451 EGLSFARAGG NWAECF-
451 RGLSFARSGG DWAECFA
449 KGLSWARSGG NWGECFS
****H   HHH      HHGTT
```

*Red letter shows the mutation site for substrate binding site.
*Bold letters are known as critical catalytic active sites.

**FIG. 12**

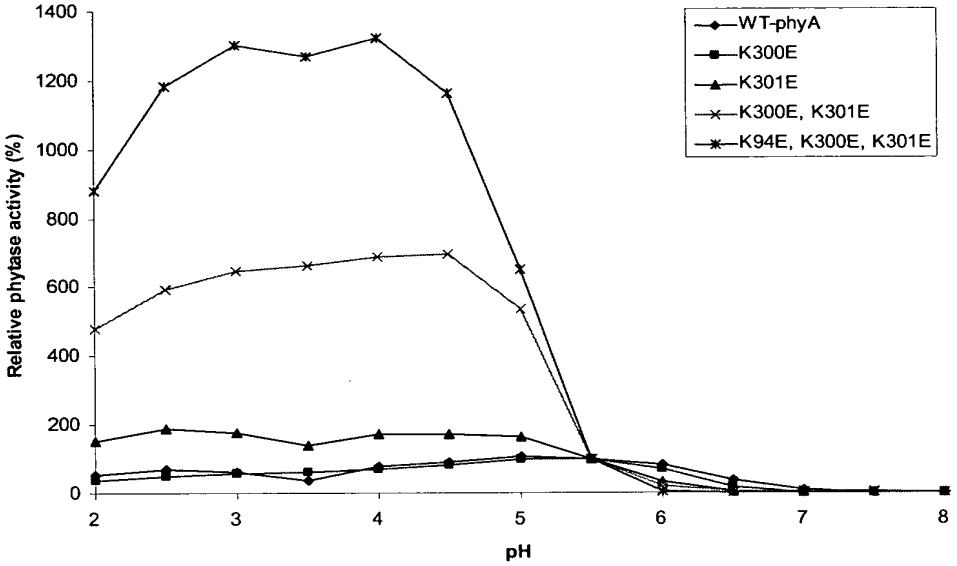
1400
1200
1000
800
600
400
200
0
Relative phytase activity (%)
2
3
4
5
6
7
8
pH
WT-phyA
K300E
K301E
K300E, K301E
K94E, K300E, K301E

*FIG. 13A*

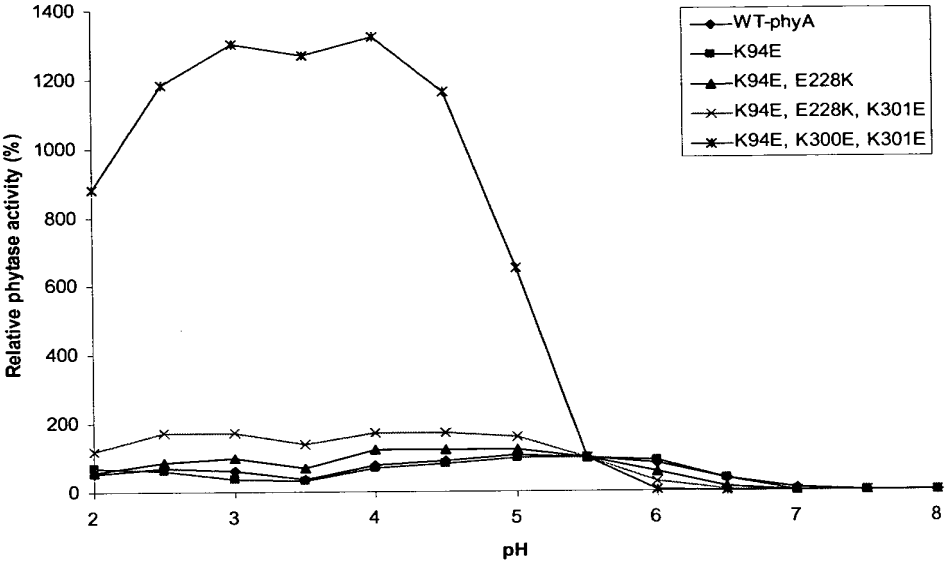
1400
1200
1000
800
600
400
200
0
Relative phytase activity (%)
2
3
4
5
6
7
8
pH
WT-phyA
K94E
K94E, E228K
K94E, E228K, K301E
K94E, K300E, K301E

*FIG. 13B*

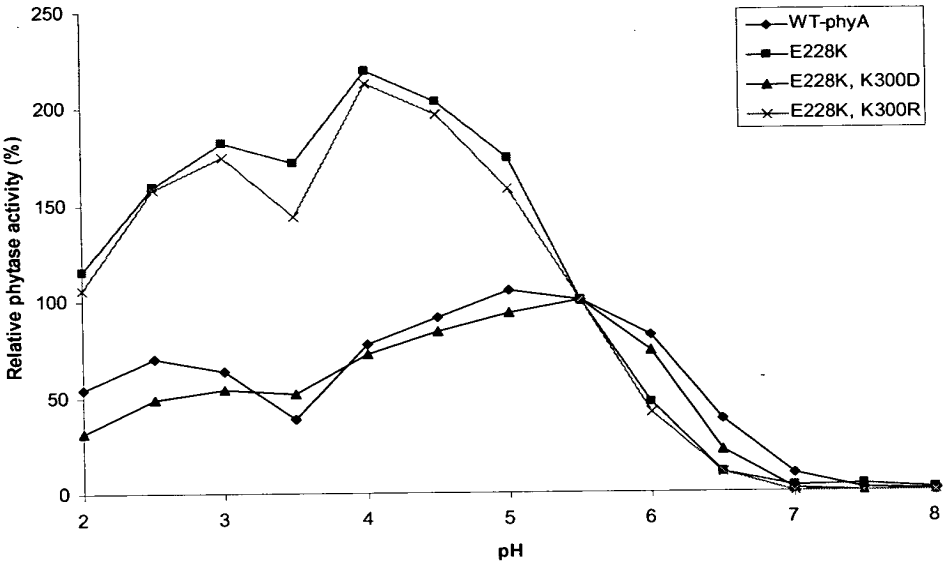
WT-phyA
E228K
E228K, K300D
E228K, K300R
Relative phytase activity (%)
250
200
150
100
50
0
2
3
4
5
6
7
8
pH

*FIG. 13C*

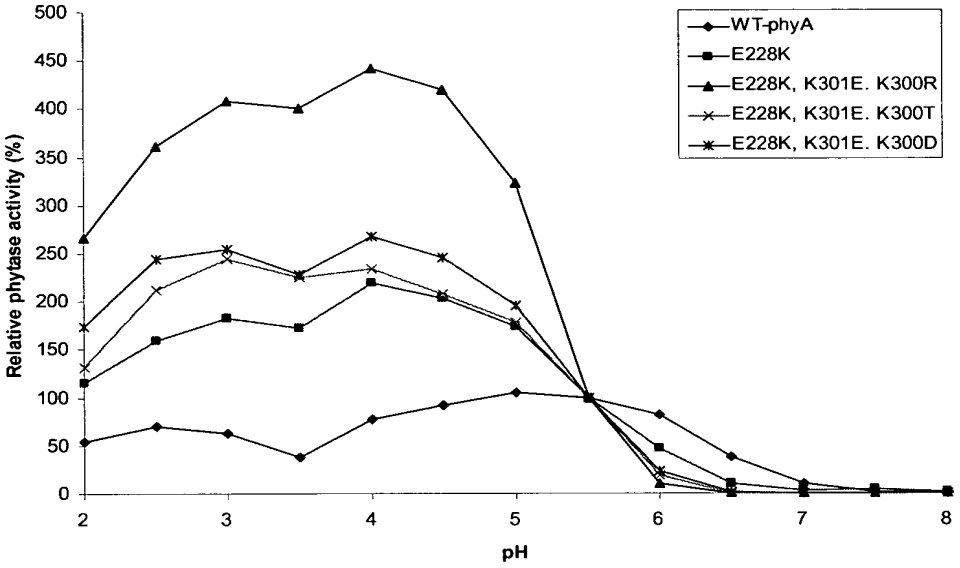
WT-phyA
E228K
E228K, K301E. K300R
E228K, K301E. K300T
E228K, K301E. K300D
Relative phytase activity (%)
500
450
400
350
300
250
200
150
100
50
0
2
3
4
5
6
7
8
pH

*FIG. 13D*

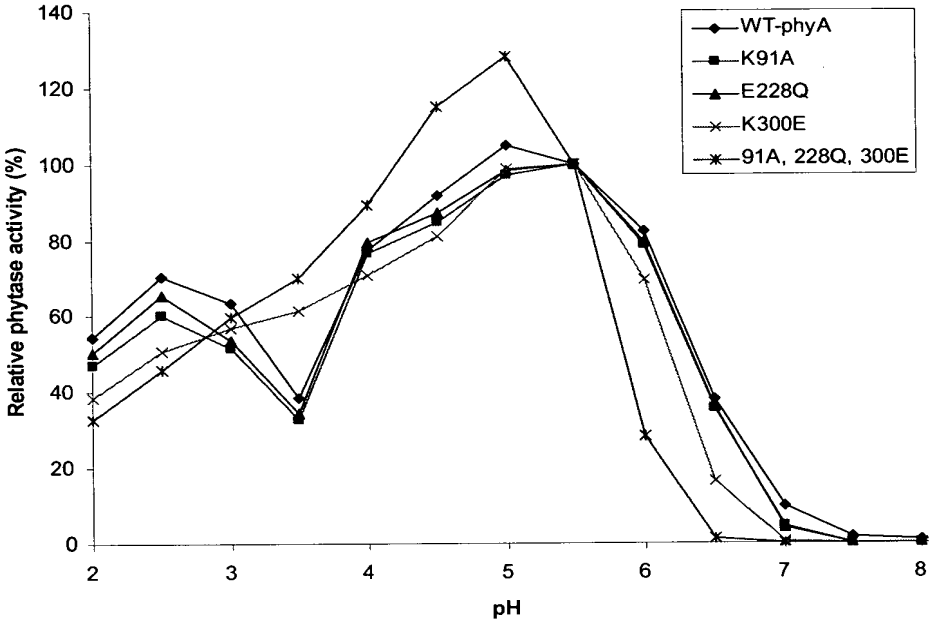
140
120
100
80
60
40
20
0
Relative phytase activity (%)
2
3
4
5
6
7
8
pH
WT-phyA
K91A
E228Q
K300E
91A, 228Q, 300E

*FIG. 13E*

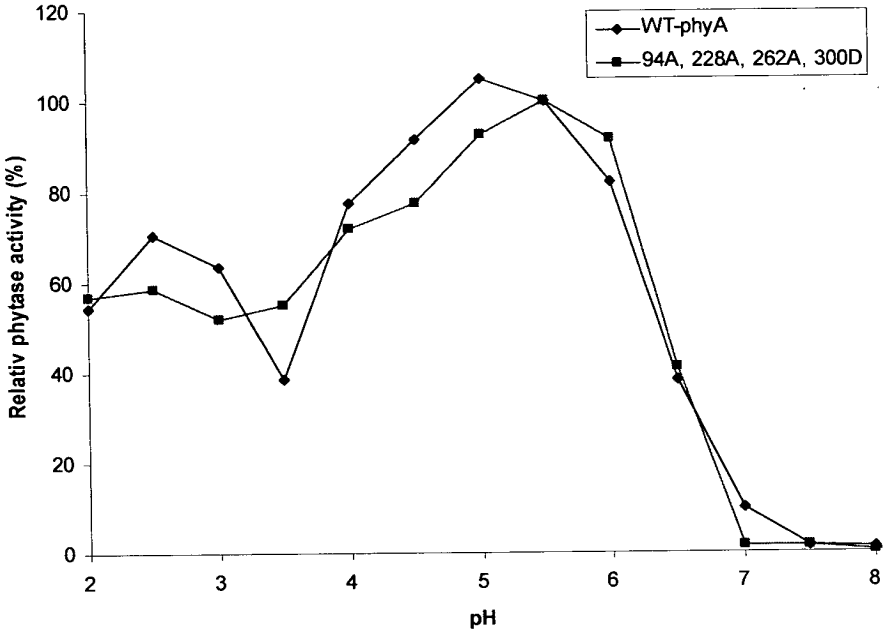
WT-phyA
94A, 228A, 262A, 300D
Relativ phytase activity (%)
120
100
80
60
40
20
0
2
3
4
5
6
7
8
pH

*FIG. 13F*

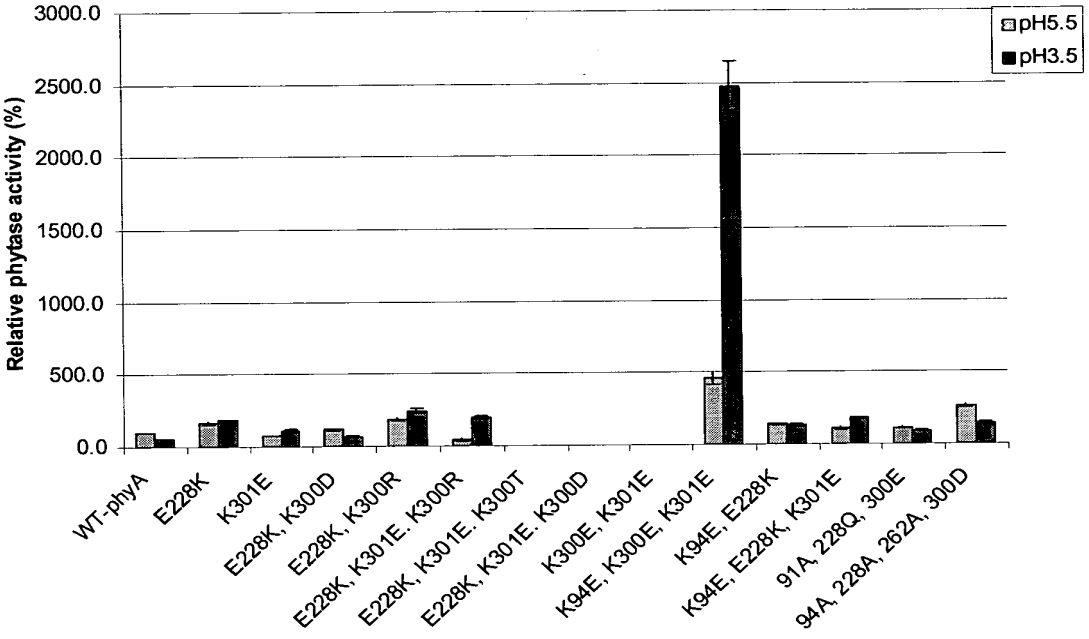
3000.0
2500.0
2000.0
1500.0
1000.0
500.0
0.0
Relative phytase activity (%)
pH5.5
pH3.5
WT-phyA
E228K
K301E
E228K, K300D
E228K, K300R
E228K, K301E. K300R
E228K, K301E. K300T
E228K, K301E. K300D
K300E, K301E
K94E, K300E, K301E
K94E, E228K
K94E, E228K, K301E
91A, 228Q, 300E
94A, 228A, 262A, 300D

*FIG. 14A*

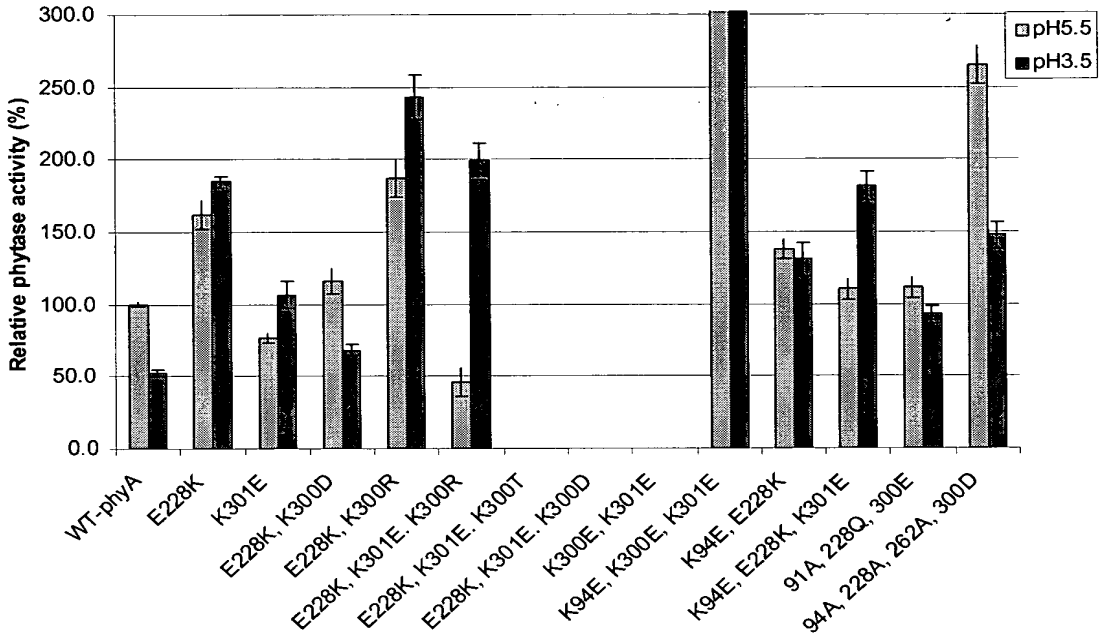
300.0
250.0
200.0
150.0
100.0
50.0
0.0
Relative phytase activity (%)
pH5.5
pH3.5
WT-phyA
E228K
K301E
E228K, K300D
E228K, K300R
E228K, K301E. K300R
E228K, K301E. K300T
E228K, K301E. K300D
K300E, K301E
K94E, K300E, K301E
K94E, E228K
K94E, E228K, K301E
91A, 228Q, 300E
94A, 228A, 262A, 300D

*FIG. 14B*

# Plasma Inorganic Phosphate (PIP) of Pigs Fed Low-P Diets

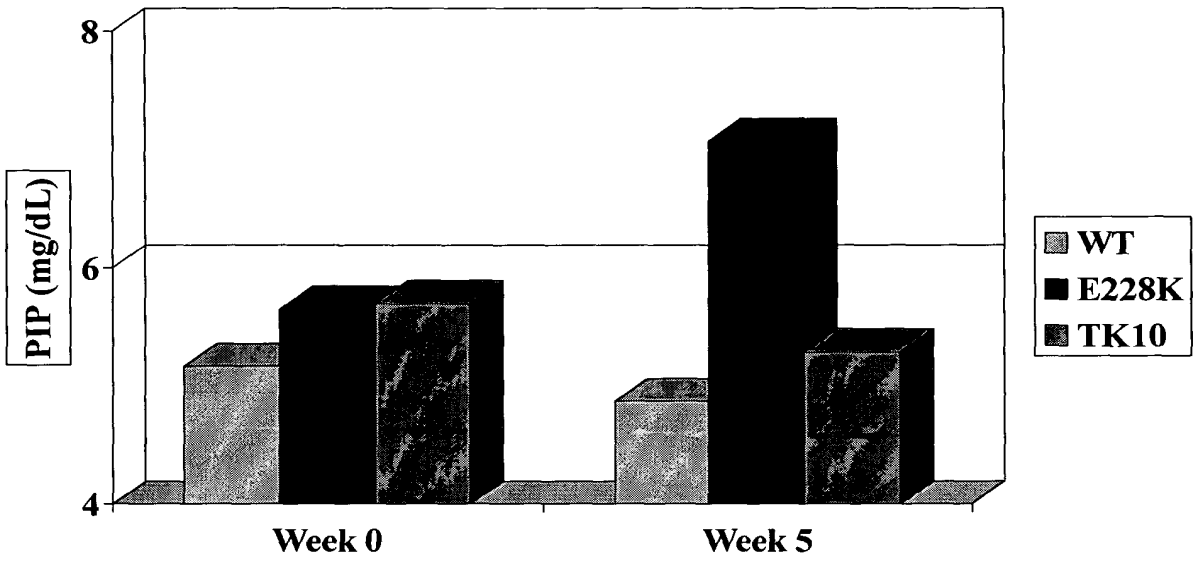


*FIG. 15*

# Plasma Alkaline Phosphatase (AKP) Activity of Pigs Fed Low-P Diets

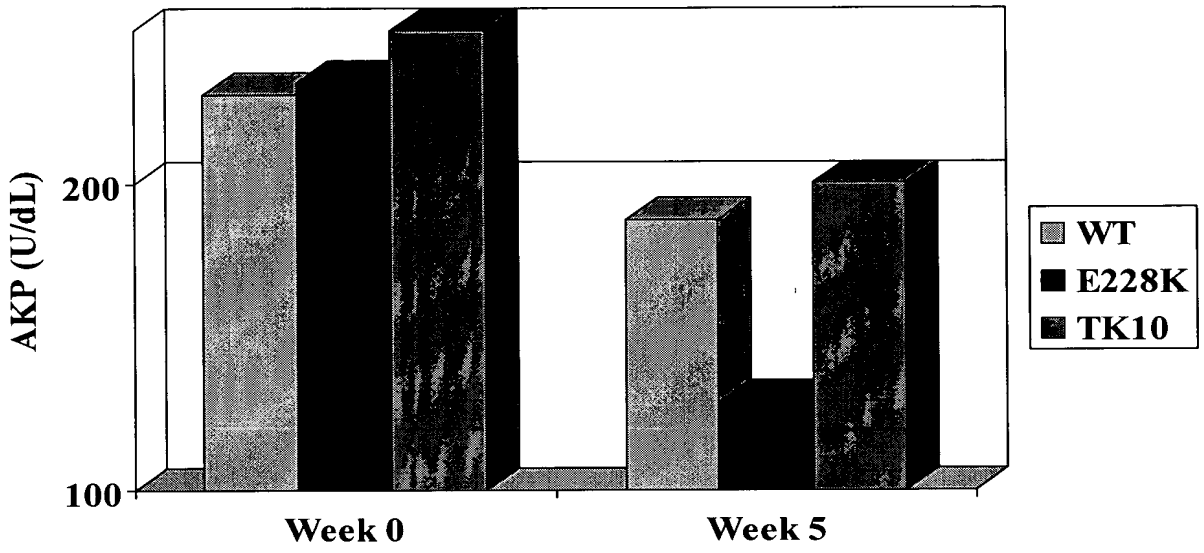


*FIG. 16*

# Average Daily Gain (ADG) of Pigs Fed Low-P Diets

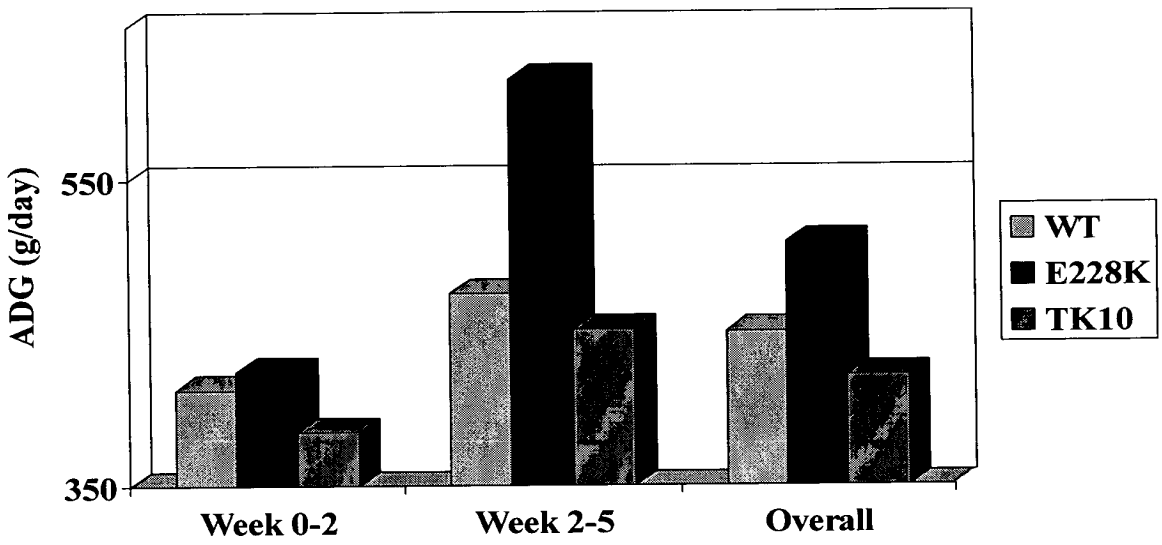


*FIG. 17*

# Gain/Feed of Pigs Fed Low-P Diets

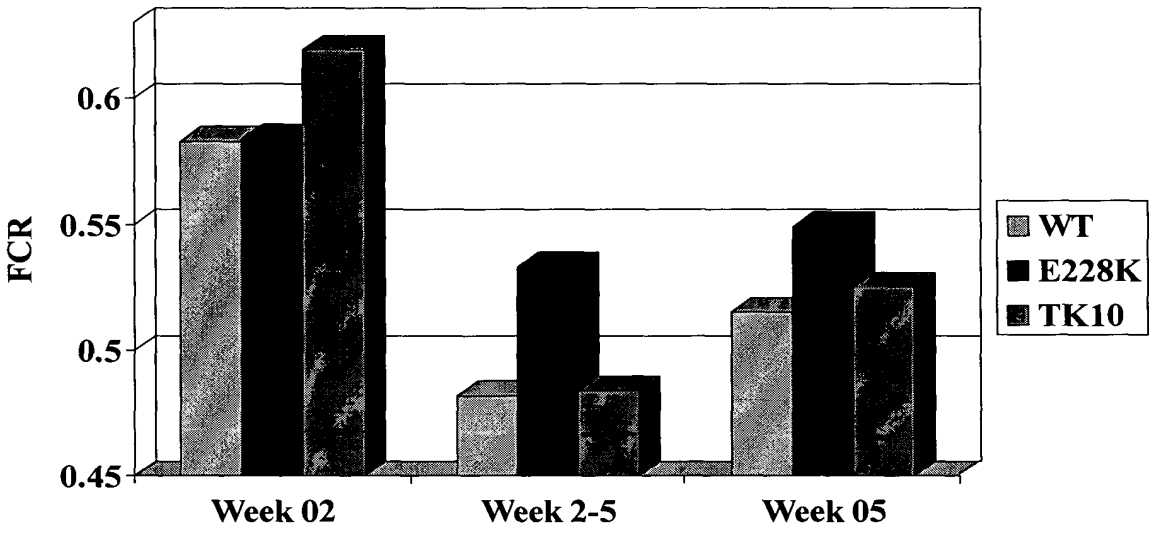


*FIG. 18*